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THE APPLICATION OF NANOTECHNOLOGY IN DEVELOPING TALAROMYCES FLAVUS FORMULATIONS

LALEH NARAGHI & MARYAM NEGAHBAN

Iranian Research Institute of Plant Protection,

Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

ABSTRACT

In Iran, during significant research, the importance of the antagonistic fungus, Talaromyces flavus has been known for the inhibition of the growth of some important plant soil pathogens. According to the results obtained to this stage of the research, the commercialization of the bioformulation of this fungus is of particular importance. Since the marketing is considered as one of the important factors for the continuation of commercialization, considering the type of bioformulation specifically nanoformulations with easy applications can greatly affect the attraction of relevant consumers and a successful marketing. The main objective of this research is increasing the stability of T. flavus using the preparation of the effective nanoformulations containing T. flavus that it can be applied in controlling plant diseases in future. In this research, four nanoformulations (two nanocapsules, one nanoemulsion and one nanopowder) containing Talaromyces flavus were prepared. For preparation these nanoformulations, the polymerization method was used. In this method, the organic phase, including plant oil and the fungal biological agent was added to the aqueous phase, including several hydrophilic monopolymers such as poly urea formaldehyde, chitosan and starch. In the next step, the cross link polyureaformaldehydekers such as calcium chloride and surfactant were added to all two phases and homogenization was carried out by homogenizer in 350C and 10000 rpm. In this study, nanoformulations were prepared in two types: nanocapsule (F1 and F3), a type of nanoemulsion (F2), and a type of powder nanoformulation (F4) of T. flavus. Therefore, in light of the recent advances in the application of nanotechnology in various sciences, it seems necessary to prepare different nanoformulations of the above biological agent with an emphasis on the ease of use, and study the efficacy in biological control of the plant diseases.

KEYWORDS: Nanoformulation, Nanopesticide, Nanotechnology, Penicillium dangeardii & Talaromyces flavus

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Original Article

INTRODUCTION

During conducted studies in Iran, desired results of *Talaromyces flavus* antagonistic fungus and other bacterial and fungal antagonistic microorganisms on controlling some of the most important soil pathogens such as *Verticillium dahliae*, *Verticilliumalbo-atrum*, *Fusarium oxysporum* and *Rhizoctonia solani* have been proven in several crops including cotton, sugar beet, potato, tomato and greenhouse cucumber have been proven (Ardekani *et al*., 2009; Shahraki *et al*., 2009; Heydari and Pessarakli, 2010; Naraghi *et al*., 2010a, b and c; Naraghi *et al*., 2011; Kakvan *et al*., 2013; Mansoori *et al*., 2013).

Also, the application of this fungus in fields as reproduced in the form of solid fermentation on the plant residues or their mixture with peat soil reduced the incidence of diseases and increased yield in these products; so that in cotton, we had 50 % reduction in the percentage of verticillin wilt disease, 37% reduction in the percentage

of the seedling death disease and 30% increase in yield; in potato we had 40% reduction in the disease percentage and 17% increase in yield (Naraghi *et al*., 2014b); in sugar beet we had 93% increase in the number of healthy seedlings and 50% increase in yield (Naraghi *et al*., 2014a); in tomato we had 27% reduction in disease severity and 23% increase in yield (Farhang Niya *e t al*., 2015) and in greenhouse cucumber we had 30% reduction in disease severity and 7% increase in yield. Since the mass production and commercialization of biological agents are a matter of marketing and attracting the attention of relevant consumers (Husen *et al*., 2006; Alimi *et al*., 2009; Kaewchai *et al*., 2009; Pereira *et al*., 2009) currently, the commercialization of the biological agent of *T. flavus* and the importance of its various bioformulations, including nanoformulations, seems essential.

In recent decades, nanotechnology has been dramatically developed in various fields of pharmacology, medicine, and agricultural chemical pesticides. The issue that necessitates research and development in the field of nano-pesticides is the pest resistance phenomenon to pesticides; therefore, the introduction of nano-pesticide to researchers will boost research and development in this relatively new field. Considering the environmental problems and costs associated with the consumption of large quantities of the conventional pesticides, as well as the problems caused by the resistance of pests to these pesticides, research, and development in the field of nanopesticide can be considered as a necessity.

The use of biodegradable polymers in producing highly efficient nano-emulsions and nanoparticles made of natural and biodegradable materials can be an effective step in this field. In order to increase the efficiency and reduce environmental hazards, encapsulated formulation is the best option (Maji *et al*., 2014). Therefore, the production of bioformulation in nano and microform provides a controlled ability, increased strength and stability, and protects the active ingredient under adverse environmental conditions such as light and moisture. Also, the use of nanoencapsulated formulations can help reduce pesticide dose used, economic savings, protect the environment and reduce its environmental hazards as well as better products' exports (Martin *et al*., 2010).

Nanoparticles have a larger surface-volume ratio than microparticles, which increases their activity level and controlled release. Also, the other advantage of nanoparticles is that they do not stimulate the human and animal body immune system and rapidly exits the body (Guan *et al*., 2008). Nanocapsule technology that contains nanoscaled fungicidal or pesticide molecules is one of the ways to produce pesticide formulations that eliminates more easily and quickly pests (Guan *et al*., 2008). An emulsion is a heterogeneous system composed of two non-mixing liquids, one of which is dispersed in droplets in another. An emulsion with a droplet size of about nanometers and typically range from 20 to 200 nm is called nanoemulsion (Ostertag *et al*., 2012). The unique structure and properties of nanoemulsions have produced many advantages in many industries compared with the conventional emulsions. Some applications of nanoemulsion systems in industries can be seen, their role in encapsulating and controlling the release of useful compounds, such as essential oils, vitamins, and etc. (Hofman and Kan, 2014).

The main aim of this study was to produce different nanoformulations containing *T. flavus* for investigating their efficacy in controlling some important plant diseases in the future studies.

MATERIALS AND METHODS

This study was begun from February in 2017 and it took longer for one year. The different steps of this research were carried out in the Iranian Research Institute of Plant Protection.

Production of Nanocapsulated Formulations Containing *Talaromyces Flavus*

The production of nanocapsules is a combination of polymerization and networking based on Guan *et al*. Method (2008) and by making changes consistent with the growth conditions of the biological fungus (changes in the amount or type of polymer, surfactants, and oils, fatty acid stirring round, and temperature). In the polymerization process, the organic phase was made up of the plant oil with a mixture of biological fungus added in an aqueous phase consisting of hydrophilic polymer monomers such as a mixture of one of two polymers of formaldehyde, originate, starch and chitosan. Subsequently, linkers such as calcium chloride, as well as surfactants and the accompanying materials, and fatty acid oils were added to both phases, and the homogenization was done at 35 °C of a homogenizer with a range of 5,000 to 10,000 rpm. Finally, polymer particles formed in the form of a capsule around the particles of the biological fungus.

Production of Nanoemulsion Formulation Containing *Talaromyces Flavus*

A self-assemble pattern was used to prepare a nanoemulsion formulation containing *T. flavus* based on Guan *et al*. Method (2008). The final formulation was an nanoemulsion in which the plant oil hydrophobic nanoparticles are formulated with a biocompatible formulation. The compounds of this formulation were: the active ingredient of the biological fungus and plant oil, such as hydrophobic castor, twin surfactant, carboxymethylcellulose viscous material, coconut moisturizer, diethanol amide fatty acid, stabilizers such as polyvinyl alcohol and linkers such as calcium chloride and biocompatible polymers such as ethylene glycol, starch and carrageenans. First, a uniform solution of biocompatible polymers was prepared, then surfactants such as toxin and the accompanying materials were added to the solution and a completely homogeneous mixture of polymer and solvent was prepared with a homogenizer with 2000 to 12000 rpm at 25 °C. Then, suspensions containing spores of the biological fungus plus plant fatty acids, oil of castor and coconut were added as drops. At the next stage, crosslinker (calcium chloride) was added in both phases to form the nanoparticles around the spores of the biological fungus. Finally, the nanoparticles were surrounded by spores of the biological fungus.

Production of Nanopowder Formulation Containing *Talaromyces Flavus*

In powdered nanoformulation, a suspension containing biological fungal spores was put in the aqueous phase, including maltodextrin, xanthan gum, fatty acid, ethanol amid and oleic acid, and then completely powdered in a homogenized with 2000 to 12000 rpm at 25 °C.

RESULTS

Qualitative and Quantitative Introduction of the Compounds used in 100 grams of Prepared Nanoformulations

In this study, four nano-formulations were made of two types of nanocapsules, one type of nanoemulsion, and one type of nanopowder (Fig. 1), all of which were used in 100 grams, qualitatively and quantitatively, as listed in Table 1.

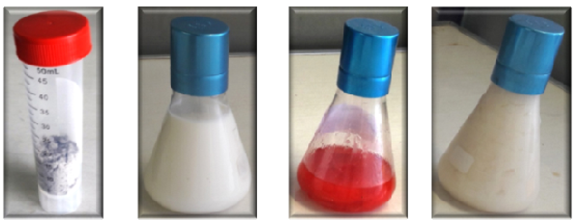

Figure 1: Prepared Nano-Formulations: (from Right to left) Nanocapsule 1(F1), Nanoemulsion (F2), Nanocapsule 2 (F3) and Nanopowder (F4)

Table 1: Types of Nanoformulations Prepared and the Amount of Compounds used Per 100 g of any Type of Nanoformulation

Nanocapsule No.1 (F1)		Nanoemulsion (F2)		Nanocapsule No.2 (F3)		Nanopowder (F4)	
Compound	Value (gr)	Compound	Value (gr)	Compound	Value (gr)	Compound	Value (gr)
Biological fungus*	19.00	Biological fungus*	35.00	Biological fungus*	53.00	Biological fungus*	5.00
Alginate 1%	8.00	Yoril alcohol	17.50	Urea	9.00	Malto Dextrin	41.50
Castor oil	16.00	Castor oil	9.00	Castor oil	4.50	starch	33.50
Coconut fatty acid	32.00	Coconut fatty acid	2.00	Coconut fatty acid	4.50	Fatty acid	8.50
Sodium chloride 0.1%	2.00	Sodium chloride 0.1%	4.00	Sodium chloride 0.1%	2.00	Xanthan Gum	8.50
		Surfactant		Surfactant	5.50		
Polyethylene glycol	20.00	Polyethylene glycol	12.00	Formaldehyde	18.50	-	-
Butanol**	3.00	Butanol**	3.00	Butanol**	3.00	Butanol**	3.00

* For the preparation of biological fungi, the suspension of *Talaromyces flavus* fungus at a concentration of 10^9 spores/ml has been used.

** Due to the preparation of nanoformulations under non-sterile conditions, in order to prevent bacterial and fungal contamination, butanol was used to investigate the efficiency of nano-formulations in inhibiting growth.

DISCUSSIONS

Protecting agricultural products against pest infestations is a matter for the government, farmers, and people specializing in this field. From ancient times, artificial pesticides are used to control pests. However, the environmental hazards of pesticides, such as rising pests to synthetic toxins, have increased the toxicity of humans, mammals, and insects by the use of low-risk pesticides. Micro-based toxins and nanomaterials are new formulas that have recently been considered as a good alternative to artificial chemical toxins. Production of nano-sized pesticide formulations effectively protects them against environmental degradation factors such as temperature, rain, UV, light, etc. By increasing the rate of solubility of pesticides in water and controlling releases, effective pesticides increase the effect of these pesticides. Data and optimize the use of poisons. Little research has been done on the production and use of micro and nano pests formulated for pest control. Nanoparticles have a larger volume of the surface than microparticles, which increases their activity level and control their release. Also, the other advantage of nanoscale particles is that these compounds do not stimulate the immune system of humans and other mammals, and quickly go out of the body (Guan *et al*., 2010).

Nanoformulations of plants' essential oil extracted against pests resistant to the conventional pesticides had more than five times potent pesticide effect (Rajakumara and Rahuman, 2011). Nanoformulations of Permethrin by evaporation of oil solvent in water could have a better effect on controlling the lobe of Culex mosquito larvae (Anjali *et al*., 2010). The degradation properties of Bacillus thuringiensis have been investigated using nanoparticles of chitosan polymer on Anopheles mosquito larvae (Zhang *et al*., 2015). Also, using dextrose and gelatin polymers biopesticide containing Beauveria, Metharhisium and Paecilomyceswas prepared against Hypothenemushampei (Niranjana *et al*., 2004).

Also *Beauveria bassiana*, the formulations containing silver nanoparticles were used for fighting larvae (Prabakaran *et al*., 2016). In a study on the effects of disinfection of barley and sunflower seeds with silver nanoparticles containing fungicide on mycorrhizal symbiosis, it was found that in the seed treatment with nanofungicides in comparison with the conventional fungicides, a significant increase was found in the amount of mineral absorption by the root and consequently a significant increase was found in the vegetative traits. Covering materials for encapsulation include gum, starch, gelatin, and polymers. Recently chitosan and phospholipids are also used. According to recent research, it seems the

use of nano-encapsulation techniques and the components in storage pest control can play an important role in increasing the efficiency and durability (Prasad *et al*., 2014).

A large number of insecticidal plant compounds are highly evaporated and susceptible to decomposition. The loading of the plant compounds into nanoparticles causes were released under control and delaying decomposition and evaporation. There are limited papers about the effect of nano-emulsion insecticides and nano-capsules loaded with pesticide-based compounds against pest insects (Margulis-Goshen and Magdassi, 2013).

There are several methods for producing nanoparticles or nanocapsules. Among them, we have the polymerization method, which is one of the quickest methods, in which continuous aqueous phase is mixed with continuous organic phase (Kirthi *et al*., 2011). Also, we have the methods of conservation and ion gelling using biodegradable hydrophilic polymers such as chitosan, gelatin, and sodium alginate, which is in fact, a mixture of two aqueous phases placed in a polymeric phase such as chitosan and sodium alginate, and in this method positive charge in a polymer with cross negative charge is mixed with the links such as sodium triphosphate or calcium chloride, and form capsules (Ebrahimnejad *et al*., 2011).

In the last 20 years, there have been significant reports on the preparation of bioformulations containing antagonistic fungi using solid and liquid fermentation and their optimization at various stages of production (Pascual *et al*., 1999; Budge and Whipps, 2001; Schuster and Schmoll, 2010; Caramez *et al*., 2012; Kakvan *et al*., 2013; Sargin *et al*., 2013). Pascual *et al*. (1999), for example, succeeded in producing solid bioformulation from *Epicoccum nigrum* fungus on wheat. After analyzing alcoholic solutions including glycerol, mannitol and arabitol on sporulation of this fungus, the most significant increase was reported in the sporulation by glycerol.

So far, overseas bioformulations have been commercially registered such as Ketomium containing *Chaetomium globosum* and *Ch. Cupreum*; Promote containing *T. harzianum* and *T. viride*; Soil Gard containing *Gliocladium virens*; Trichodex containing *T. harzianum*, *Pisolithus tinctonus* and *Glomus intraradices*; Trichodermin containing *T. harzianum* and Protus WG containing *Talaromyces flavus* (Merwel *et al*., 1974; Koch, 1999; Kaewchai *et al*., 2009). In Iran, in order to use *T. flavus* fungus in the greenhouse and field, bioformulations containing it have been prepared using solid substrates including worm fertilizer, wheat straw, wheat bran, corn wood, rice bran, wheat straw combined with wheat bran, perlite mixed with sugar supplement, and peat soil mixed with sugar supplementation. The best substrate in terms of the efficiency in increasing sporulation and stability for *T. flavus* isolate related to cotton and potato was rice bran. However, the most effective substrate in increasing sporulation and stability for *T. flavus* isolated related to tomato and greenhouse cucumber was peat soil mixed with sugar supplements (Naraghi *et al*., 2010a; Naraghi *et al*., 2010b; Naraghi *et al*., 2010c).

The application of rice bran substrate for *T. flavus* reproduction has been reported in the field of biological control of garlic white rot and its vegetative traits' reinforcement through the use of this fungus. Also, for biological control of the seedling death and root rot in cotton and cucumber, this substrate has been used for the reproduction of antagonistic bacteria, such as Pseudomonas fluorescens (Ardekani *et al*., 2010; Khabbaz and Abbasi, 2014; Mehdizadehnaraghi *et al*. 2015).

The results of greenhouse experiments in the field of biological control with Verticillium wilt in greenhouse potato, tomato and cucumber caused by *V. Albo-atrum* by bioformulations containing different isolates of *T. flavus* showed that these isolates reduced the disease index and increased vegetative traits such as the root length, crown length, height, fresh weight and dry weight of the above plants significantly (Naraghi *et al*., 2010a, 2010b and 2010c; Naraghi *et al*.,

2012a and 2012b). Also, field studies were conducted on the possibility of biological control of cotton verticillium wilt disease and seedling death; sugar beet seedling death; potato verticillium wilt and greenhouse cucumber and tomato Fusarium wilt using *T. flavus* bioformulation. The results showed that the use of this bioformulation, in addition to the significant reduction of the disease index, has also led to a significant increase in yield (Naraghi *et al*., 2014a and 2014b; Farhang Niya *et al*., 2015).

So far, there have been two reports in the field of medicine and agriculture for the preparation of nano-fungicides-from the studied fungus genus Penicillium in the present study (Priyadarshini *et al*. 2014; Khan and Jameel, 2016). In the field of medicine, *Penicillium fistula* has been used to prepare nano fungicides against *Candida albicans*, and in the crop fields, for the preparation of nano fungicides against some fungal pathogens of the plant diseases, *Talaromyces flavus* (Anamorph: *Penicillium dangeardii*) has been used.

CONCLUSIONS

The overall results of this study showed that the use of biodegradable polymers in the production of highly efficient nanoemulsions and nanocapsules made of natural and biodegradable materials such as essential oil of medicinal herbs can be an effective step in this field. The use of nano-pesticides, the production, application and environmental considerations indicate that research and development can be effective in reducing pest resistance phenomena to pesticides. Nano-formulations prepared by microorganisms, such as bacteria and fungi, are used in the biological struggle against insects and facilitate the entry of agents to insects' body. Nanoformulations of Pirimphospesticide make the formulation well-kept in the dark and more stable.

The novelty of this research is the introducing nanotechnology application for increasing the stability of *T. flavus*. The fungal biological agent, *T. flavus* are without resting and resistance structures. This subject causes that *T. flavus* cannot remain in the soil fields for considerable periods. Therefore, it is favorite that the technique is applied for increasing the stability of *T. flavus*. By preparing the nanoformulations containing *T. flavus* and producing the spores coated with nanoparticles, this fungus gets, the more stability that it causes increased its efficacy in controlling plant diseases.

REFERENCES

1. *Alimi, T., Ajewole, O. C., Olubode-Awosola, O. O., and Idowu, E. O. 2006. Economic rationale of commercial organic fertilizer technology in vegetable production in Osun State of Nigeria. Journal of Applied Horticulture, 8:2: 159-164.*
2. *Anjali, C. H., Sudheer Khan, S., Margulis Goshen, K., Magdassi, S., Mukherjee, A., and Chandrasekaran, N. 2010. Formulation of waterdispersible nanopermethrin for larvicidal applications. Ecotoxicology and Environmental Safety. 73:8: 1932-1936.*
3. *Ardakani, S. S., Heydari, A., Khorasani, N., and Arjmandi, R. 2009. Development of new bioformulations of Pseudomonas fluorescens and evaluation of the products against damping-off of cotton seedlings. Journal of Plant Pathology, 2: 1: 83-88.*
4. *Budge, S. P., and Whipps, J. M. 2001. Potential for integrated control of Sclerotinia sclerotiorum in glasshouse lettuce using Coniothyrium minituns and reduced fungicide application. Phytopathology, 91:2: 221-227.*
5. *Caramez, M., Damaso, T., Costaterzi, S., Farias, A. X., Pereira de Oliveira, A. C., Fraga, M. E., and Couri, S. 2012. Selection of cellulolytic fungi isolated from diverse substrates. Brazilian Archives of Biology and Technology, 55:4: 513-520.*
6. *Ebrahimnejad P, Dinarvand R, Jafari MR, Tabasi SAS, Atyabi F. 2011. Characterization, blood profile and biodistribution properties of surface modified PLGA nanoparticles of SN-38. International Journal of Pharmaceutics. 406:1,2: 122-127.*

7. Engelelkes, C. A., Nuclo, R. L., and Fravel, D. R. 1997. Effect of carbon, Nitrogen, and C:N ratio on growth, sporulation, and biocontrol efficacy of Talaromyces flavus. Phytopathology, 87: 500-505.
8. Farhang Niya, S., Naraghi, L., Ommati, F., Pirnia, M. 2015. Evaluation of the efficacy of the biological compound affected by Talaromyces flavus in controlling tomato Fusarium wilt disease in the field conditions. International Journal of Agricultural Science and Research, 5: 2: 153-164.
9. Guan H, Chi D, Yu J, Li X. 2008. A novel photodegradable insecticide: Preparation, characterization and properties evaluation of nano-Imidacloprid. pestic biochem physiology. 92:2: 83-91.
10. Guan, Ch., and Yu., J. 2010. Dynamics of residues from a novel nano-imidacloprid formulation in soybean fields. Plant Protection, 29:9: 942-946.
11. Hammoudi, O., Salman, M., Abuamsha, R., Ehlers, R. 2012. Effectiveness of bacterial and fungal isolates to control phoma lingam on oilseed rape, Brassica napus. American Journal of Plant sciences, 3:1: 773-779.
12. Husen, E., Simanungkalit, R. D. M., Suraswati, R., and Irawan, I. 2007. Characterization and quality assessment of Indonesian commercial biofertilizer. Indonesian Journal of Agricultural Science, 8:1; 31-38.
13. Johnson, k. B., Dileone, J. A. 1999. Effect of antibiosis on antagonist dose-plant disease response relationships for the biological control of crown gall of tomato and cherry. Phytopathology, 89:1: 974-980.
14. Kaewchai, S., Soytong, K., and Hyde, K. D. 2009. Mycofungicides and fungal biofertilizers. Fungal Diversity, 38: 25-50.
15. Naraghi, Laleh, et al. "Assessment of Genetic Diversity in Different Isolates of Talaromyces Flavus by Rapd Molecular Marker." International Journal of Agricultural Science and Research (IJASR) 4.6 (2014): 53-60.
16. Kakvan, N., Heydari, A., Zamanizadeh, H. R., and Naraghi, L. 2013. Development of new bioformulations using Trichoderma and Talaromyces fungal antagonists for biological control of sugar beet damping-off disease. Crop Protection, 53: 1: 80-84.
17. Kah, M. and Hofmann, T. 2014. Nanopesticide research: current trends and future priorities. Environment international, 63: 224-235.
18. Khabbaz, S. E., and Abbasi, P. A. 2014. Isolation characterization and development of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. Canadian Journal of Microbiology, 60: 1: 25-33.
19. Khan, N. T., and Jameel, N. 2016. Antifungal activity of silver nanoparticles produced from fungus, Penicillium fellutanum at different pH. Journal of Microbial and Biochemical Technology, 8:5: 440-443.
20. Singh, Jagmeet, Jaspal Singh, and Manpreet Kaur. "Applications of Nanotechnology in Construction Industry."
21. Kirthi AV, Rahuman AA, Rajakumar G, Marimuthu S, Santhoshkumar T, and Jayaseelan, C. 2011. Acaricidal, pediculocidal and larvicidal activity of synthesized ZnO nanoparticles using wet chemical route against blood feeding parasites. Parasitology Research, 109:2: 461-472 .
22. Koch, E. 1999. Evaluation of commercial products for microbial control of soil-borne plant disease. Crop Protection, 18:2: 119-125.
23. Maji, R., Dey, N., Satapathy, B., Mukherjee, B. and Mondal, S. 2014. Preparation and characterization of Tamoxifen citrate loaded nanoparticles for breast cancer therapy. International journal of nanomedicine, 9: 3107.
24. Mansoori, M., Heydari, A., Hassanzadeh, N., Rezaee, S., and Naraghi, L. 2013. Evaluation of Pseudomonas and Bacillus bacterial antagonists for biological control of cotton Verticillium wilt disease. Journal of Plant Protection Research, 53:1: 154-156.

25. *Margulis-Goshen, K. and Magdassi, S. 2013. Nanotechnology: an advanced approach to the development of potent insecticides. Advanced Technologies for Managing Insect Pests. (ed.), pp. 295-314. Springer.*

26. *Martín, Á., Varona, S., Navarrete, A. and Cocero, M. 2010. Encapsulation and co-precipitation processes with supercritical fluids: applications with essential oils. The Open Chemical Engineering Journal, 4:1: 31-41.*

27. *Mehdizadehnaraghi, R., Hydrae, A., Zamanizadeh, H. R., Rezaee, S., and Nikandam, J. 2015. Promotion of garlic growth characteristics using bioformulations developed based on antagonistic fungi. International Journal of Agriculture and Crop Sciences, 8:5: 654-658.*

28. *Merwel, c., Hansen, B. S., Maurice, H., and Vaughan, J. R. 1974. Mechanism of action of the mycotoxin Trichodermin, a 12,13-Epoxytrichothecene. Proceedings of the National Academy of Sciences of the united states of America, 71: 713-717.*

29. *Naraghi, L., Arjmandian, A., Heydari, A., Sharifi, K., and Afshari Azad, H. 2014a. A comparison between carbendazim fungicide and Talaromyces flavus in controlling Verticillium wilt of potato under field conditions. International Journal of Agricultural Science and Research, 4: 1: 89-100.*

30. *Naraghi, L., Heydari, A., Hesan, A., and Sharifi, K. 2014b. Evaluation of Talaromyces flavus and Trichoderma harzianum in biological control of sugar beet damping-off disease in the greenhouse and field conditions. International Journal of Agricultural Science and Research, 4: 1: 65-74 .*

31. *Naraghi, L., Heydari, A., Rezaee, S., Razavi, M., and Afshari-Azad, H. 2010a. Biological control of greenhouse cucumber Verticillium wilt disease by Talaromyces flavus. Phytopathologia Mediterranea, 49: 321-329.*

32. *Naraghi, L., Heydari, A., Rezaee, S., Razavi, M., and Jahanifar, H. 2010b. Study on antagonistic effects of Talaromyces flavus on Verticillium albo-atrum, the causal agent of potato wilt disease. Crop Protection, 29: 7: 658-662.*

33. *Naraghi, L., Heydari, A., Rezaee, S., Razavi, M., Jahanifar, H., and Mahmoodi Khaledi, E. 2010c. Biological control of tomato Verticillium wilt disease by Talaromyces flavus. Journal of Plant Protection Research, 50: 3: 360-365.*

34. *Naraghi, L., Heydari, A., Rezaee, S., and Razavi, M. 2012a. Biocontrol agent Talaromyces flavus stimulates the growth of cotton and potato. Journal of Plant Growth Regulation, 31: 471-477.*

35. *Naraghi, L., Heydari, A., Rezaee, S., Razavi, M., and Afshari-Azad, H. 2012b. Promtion of growth charasteristics in greenhouse cucumber and tomato by Talaromyces flavus. International Journal of Agricultural Science and Research, 2: 3: 129-141.*

36. *Ostertag, F., Weiss, J., McClements, D. J., 2012. Low-energy formation of ediblenanoemulsions: factors influencing droplet size produced by emulsion phaseinversion. J. Colloid Interface Sci. 388, 95–112.*

37. *Pascual, S., Melgarejo, P., and magan, N. 1999. Production of the fungal biocontrol agent Epicoccum nigrum by solid substrate fermentation: effect of water activity on accumulation of compatible solutes. Mycopathologia, 146: 1: 83-89.*

38. *Pereira, I., Ortegu, R., Barrientus, L., Moya, M., Reyes, G., and Kramm, V. 2009. Development of a biofertilizer based on filamentous nitrogen- fixing cyanobacteria for rice crops in Chile. Journal of Applied Phycology, 21: 1: 135-414.*

39. *Prabakaran, K., Ragavendran, C. and Natarajan, D. 2016. Mycosynthesis of silver nanoparticles from Beauveria bassiana and its larvicidal, antibacterial, and cytotoxic effect on human cervical cancer (HeLa) cells. An international journal to further the chemical sciences. 6: 44972-44986.*

40. *Prasad, R., Kumar, V. and Prasad, K. S. 2014. Nanotechnology in sustainable agriculture: present concerns and future aspects. African Journal of Biotechnology, 13: 6: 705-713.*

41. *Priyadarshini, E., Pradhan, N., Behari Sukla, L., Kumar Panda, P., and Kanta Mishra, B. 2014. Biogenic synthesis of floral-shaped gold nanoparticles using a novel strain, Talaromyces flavus. Annals Microbiology, 64:3: 1055-1063.*

42. *Rajakumar G, Abdul Rahuman A. 2011. Larvicidal activity of synthesized silver nanoparticles using Eclipta prostrata leaf extract against filariasis and malaria vectors. Acta Tropica. 118:3: 196-203.*

43. *Schuster, A., and Schmoll, M. 2010. Biology and biotechnology of Trichoderma. Applied Microbiology and Biotechnology, 87:3: 787-799*

44. *Shahraki, M., Heydari, A., and Hasanzadeh, N. 2009. Investigation of antibiotic, siderophore, volatile metabolites production by Bacillus and pseudomonas bacteria. Iranian Journal of Biology, 22:1: 71-84.*

45. *Heydari, A., and Pessarakli M. 2010. A review on biological control of fungal plant pathogens using microbial antagonists. Journal of Biological Sciences, 10:1: 272-290.*

46. *Zhang, Q., Hua, G. and Adang, M. J. 2015. Chitosan/DsiRNA nanoparticle targeting identifies AgCad1 cadherin in Anopheles gambiae larvae as an in vivo receptor of Cry11Ba toxin of Bacillus thuringiensis subsp. Jegathesan. Insect Biochemistry and Molecular Biology, 60:1: 33-38.*